



May 14, 2004

TO: See Distribution List

FROM: Martha Lamont, Director  
Monitoring Programs Office

SUBJECT: Microbiological Data Program Plan, July through December 2004

This Program Plan serves as the current Statement of Work for the period July 1, 2004 through December 31, 2004 for each State participating in the Microbiological Data Program (MDP). This document also stipulates work assignments for the Federal facility participating in MDP.

## **I. ADMINISTRATIVE UPDATES**

Program participants are reminded to keep MDP management informed of any critical equipment purchases, staffing issues, or expected increases in rent or sample turn-around-time (e.g., due to laboratory or office renovation/relocation). This information is required under the terms of the MDP Cooperative Agreements (Section II, Responsibilities) between USDA and participating States.

The MDP Progress Update and 2002 Data Summary has been posted on the MDP website. Data for 2003 are being compiled and the accompanying text is being drafted.

### **A. Personnel**

Linda Jo Dyer has been selected to take over the Sampling Manager's responsibilities for MDP. Jo was hired as a scientist to support the water testing program as well as MDP. Sharon Williams will continue with her current assignment as Sampling Assistant, keeping track of samples (including sampling receipt forms), preparing shipping charts, sampling schedules, updating the database site code tables, and other budget support responsibilities.

### **B. Financial/Cooperative Agreements**

MDP Cooperative Agreements for Fiscal Year 2004 (FY04) are in place. MDP anticipates that MDP FY05 funding may be established at \$5.57 million, the same level as FY 2004.

### **C. MDP Program Meetings**

The Federal/State MDP meeting will be held June 22-24, 2004, in Fairfax, VA. Managers for administrative, sampling, technical, and quality assurance (QA) functions are required to attend the meeting. Staff from MPO and USDA Headquarters will also be in attendance. Guest speakers will include Bob Brackett, FDA; David White, CDC; and Jim Kaper, University of Maryland. The following vendors will be available at break times but will not be present during the meeting discussions: Advanced Analytical, BioControl, DRG, Dynal, and Pathatrix.

### **D. Electronic Transfer of Data**

RDE System Architecture: The reengineered RDE system is a centralized system, where all RDE database files and support software will reside in Washington, D.C. and laboratory users will require only an Internet web browser on the front-end. A stand-alone Sample Information Form (SIF) data entry system for laptop/desktop computers and for PDAs (Pocket PCs) was developed to allow the capture of SIF data electronically by sample collectors. The SIF data entry system can also be used by laboratories to perform off-line data entry of paper SIF information that can then be imported into the central RDE system. Laboratories that import complete data sets into the Web-based RDE system can request that HQ-MPO forward all incoming e-SIFs to the lab for import into the lab internal database or LIMS.

RDE Version Upgrade Installed: An upgraded version of the Web-based Remote Data Entry (RDE) system was installed on April 19, 2004. This new version includes a new feature to automatically assign a unique Isolate Number to each isolate that is sent to a reference lab for further testing. Other significant modifications include a new provision to store default findings for non-detects that will pre-populate those values on new analytical results and a new "Change All Fields" function for populating all analytical result fields in the current Group for the selected organism to match the current record's values. There were also fixes for several identified and reported glitches. This upgrade and all future system modifications will be programmed by the MPO staff. MPO is maintaining a database of all user change requests, including problems and suggestions received by telephone and e-mail.

New RDE Secure Web Address: RDE users in the laboratories should be using the SSL (Secure Socket Layer) site address to access the Web-based RDE system. The SSL address is <https://www.ams.usda.gov/rde>. The only difference is the addition of the letter "s" following "http". This SSL technology is used to encrypt all data passed between the user's computer and the central web server.

## **E. PROGRAM SAMPLING AND TESTING UPDATES**

- A. Sampling Changes and Rotations:** Shipping Charts are distributed quarterly to Sampling Managers by MPO.

**Sampling Deletions:** Celery ends *June 30, 2004*.

### **Sampling Additions:**

**Green onions** will be added *July 1, 2004*. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

**Cilantro/parsley** will be added *July 1, 2004*. Each will be sampled at half monthly sampling rates. For example, if a commodity is normally scheduled for monthly collection at six sites, cilantro will be collected at three sites and parsley will be collected at three sites on the same date. If a commodity is normally scheduled for collection of seven site samples, cilantro will be collected at three sites and parsley will be collected at three sites on the same date.

Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

**Sampling Changes: Leaf and romaine lettuce** will be combined as one commodity beginning *July 1, 2004*. Either variety may be sampled on a given lettuce sampling date. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

**Sampling Continuations:** Tomatoes and cantaloupe will continue. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

**Tentative Sample Redirection:** Due to extensive laboratory renovations, some samples collected by California may be redirected to other laboratories during this timeframe. Tentative changes include: green onion samples collected by California during July through October will be sent to the Florida laboratory (FL4), cilantro/parsley samples collected by California during July through October will be sent to the Ohio laboratory (OH4), and lettuce samples collected by California during September will be sent to NSL (US4). A final determination of these proposed changes is expected no later than *June 14, 2004*.

## **B. Testing**

**Testing Deletions:** Celery ends *June 30, 2004*.

### **Testing Additions:**

**Green onions** will be added *July 1, 2004*. Samples collected in Maryland will be tested by the Ohio laboratory (OH4) and those collected in Texas will be analyzed by NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

**Cilantro/parsley** will be added *July 1, 2004*. Each will be sampled at half monthly sampling rates. For example, if a laboratory normally receives six site samples per month per commodity, three cilantro site samples and three parsley site samples will be collected on the scheduled sampling date. If a laboratory normally receives seven site samples per month per commodity, three cilantro site samples and three parsley site samples will be collected on the scheduled sampling date.

Samples collected in Maryland will be tested by the Ohio laboratory (OH4) and those collected in Texas will be analyzed by NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

**Testing Changes: Leaf and romaine lettuce** will be combined as one commodity beginning *July 1, 2004*. Either variety may be sampled on a given lettuce sampling date. Samples collected in Maryland will be tested by the Ohio laboratory (OH4) and those collected in Texas will be tested by NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

**Testing Continuations:** Tomatoes and cantaloupe will continue. Samples collected in Maryland will be tested by the Ohio laboratory (OH4) and those collected in Texas will be tested by NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

**Tentative Sample Redirection:** Due to extensive laboratory renovations, some samples analyzed by the California laboratory (CA4) may be redirected to other laboratories during this timeframe. Tentative changes include: green onion samples collected by California during July through October will be tested by the Florida laboratory (FL4), cilantro/parsley samples collected by California during July through October will be tested by the Ohio laboratory (OH4), and lettuce samples collected by California during September will be tested by NSL (US4). A final determination of these proposed changes is expected no later than *June 14, 2004*.

## **New Commodities Test Procedures:**

Green onions and cilantro/parsley will be washed using an equal weight of buffered peptone water containing 0.1% Tween 80. Standard Operating Procedure (SOP) MDP-LABOP-02 will be modified accordingly for July 1, 2004 distribution.

Method performance (validation) requirements will be stipulated in a method try-out protocol that will be supplied under separate cover. All commodities are required to undergo this try-out prior to any sample analyses. Laboratories are urged to complete the try-out prior to the June 22-24, 2004 Federal/State Meeting.

## **Target Microorganisms**

MDP laboratories will continue quantitative testing of all samples for *E. coli* using the MPN ColiComplete LST-MUG based method. Method procedures are detailed in SOP MDP-MTH-01, *Escherichia coli* MPN Method.

MDP laboratories will continue to test all samples for *Salmonella* (presence or absence) by BAX. Method procedures are detailed in SOP MDP-MTH-04, BAX<sup>®</sup> System for Detection of *Salmonella* in Fresh Produce.

MDP laboratories will continue to test all samples for *E. coli* O157:H7 (presence or absence) by BAX. Method procedures are detailed in SOP MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX<sup>®</sup> System. Presumptive positives are subjected to immunomagnetic separation (IMS) procedures and confirmed culturally, as described in SOP MDP-MTH-06, *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Confirmation.

## **C. Quality Assurance**

### **Proficiency Testing Program**

Quality Management, UK, will provide proficiency test sets for *E. coli* during June 2004. Lyophilized cultures will be sent to the AMS National Science Laboratory (NSL) and distributed to all program laboratories. Cultures shall be reconstituted and diluted per MPO instructions by the onsite Quality Assurance Unit (QAU). Samples will then be inoculated by the QAU and issued to the technical group for analysis.

## **SOPs**

SOPs are posted to the MDP website when distributed to program participants.  
<http://www.ams.usda.gov/science/MPO/SOPs.htm>.

### ***The following SOPs were distributed March 15, 2004:***

MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX<sup>®</sup> System, Original  
MDP-MTH-06, *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Confirmation, Original

### ***The following SOPs were distributed May 15, 2004:***

MDP-LABOP-08, Procedure for Testing and Maintenance of Control Strains, Original  
MDP-QA-03, Quality Assurance (QA) Controls, Original

### ***The following SOPs are planned for June 1, 2004 distribution:***

MDP-MTH-01, *Escherichia coli* MPN Method, Revision 4  
MDP-MTH-04, BAX<sup>®</sup> System for Detection of *Salmonella* in Fresh Produce, Revision 1  
MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX<sup>®</sup> System, Revision 1

### ***The following SOPs are planned for June 15, 2004 distribution:***

MDP-SHIP-03, Procedures for Packaging, Shipping, and Archiving Microbiological Cultures, Original  
MDP-LABOP-02, Sample Receipt and Elution Procedure, Revision 4

### ***The following SOPs are planned for archival:***

MDP-LABOP-07, Maintenance of *Salmonella* and *E. coli* Positive Control Cultures with GFP Plasmid, Original, Archived May 15, 2004  
MDP-SHIP-01, Procedures for Packaging and Shipping Microbiological Cultures, Original, Archive June 15, 2004  
MDP-SHIP-02, MDP Microbiological Cultures Shipping Instructions, Original, Archive June 15, 2004

## **D. Archiving and Additional Testing**

### **Archival of Isolates**

The AMS NSL, Gastonia, NC has been established as a centralized location for archival of isolates as well as a distribution center for isolates from the laboratories to the reference laboratories.

### **Additional Testing by Reference Laboratories**

*E. coli* LST broth from samples with presumptive positive results by the MPN method is shipped directly to the Florida laboratory for multiplex PCR (mPCR). The LST mixed culture is tested for shiga toxin producing *E. coli* (STECs), enterohemorrhagic *E. coli* (EHECs), and enterotoxigenic *E. coli* (ETECs). Microbank™ vials of isolates are then shipped to NSL.

For isolates from all other methods, laboratories freeze organisms in two Microbank™ vials which are shipped to NSL.

Isolates are shipped from NSL to reference laboratories as follows:

- All *E. coli* isolates are shipped to the laboratory at Pennsylvania State University for serotyping and to the FDA/CVM laboratory in Laurel, MD for antimicrobial resistance testing and inclusion in the National Antimicrobial Resistance Monitoring System (NARMS) and pulsed field gel electrophoresis (PFGE) analysis for inclusion in PulseNet.
- Isolates from all other methods are shipped to FDA/CVM for antimicrobial resistance testing, PFGE and inclusion in PulseNet, and serotyping.

### **Data Transfer**

AMS will transfer data to CDC and FDA on a semi-annual basis.

## **E. Training**

MPO is working with FDA to implement a week-long training course for MDP laboratory personnel September 20-24, 2004. This hands-on course will be held in the Florida laboratory (FL4) and will cover microbiological, molecular biological, and immunological methods used in MDP, as well as troubleshooting problems experienced by the laboratories.

## **F. Future Program Directions**

**Sprouts** are being investigated as an additional commodity for 2005. Alfalfa/clover sprouts will be tested for all required organisms. Routine analyses of all samples are planned to be concentrated in two MDP laboratories.

**New wash methods** are being investigated for all commodities. In order to increase organism recovery, alternative preparation methods are under investigation including use of a universal pre-enrichment broth (UPB) and an overnight soak for several of the commodities. Tomatoes may require crushing in order to effectively recover target organisms.

**Multiplex PCR** is currently performed by the Florida laboratory on all program *E. coli* LST broth presumptive positives. This technology will be transferred to all program laboratories in the future.

***Shigella***: MDP will continue to work on the implementation of *Shigella* testing for all commodities, tentatively *October 1, 2004*. The FDA protocol, including the enrichment step will be used. DNA cleanup procedures and initial method performance studies will be investigated by the Minnesota laboratory.